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# Parasitic Nematode-Induced Modulation of Body Weight and Associated Metabolic Dysfunction in Mouse Models of Obesity

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**Obesity is associated with a chronic low-grade inflammation characterized by increased levels of proinflammatory cytokines that are implicated in disrupted metabolic homeostasis. Parasitic nematode infection induces a polarized Th2 cytokine response and has been explored to treat autoimmune diseases. We investigated the effects of nematode infection against obesity and the associated metabolic dysfunction. Infection of RIP2-Opa1KO mice or C57BL/6 mice fed a high-fat diet (HFD) with *Nippostrongylus brasiliensis* decreased weight gain and was associated with improved glucose metabolism. Infection of obese mice fed the HFD reduced body weight and adipose tissue mass, ameliorated hepatic steatosis associated with a decreased expression of key lipogenic enzymes/mediators, and improved glucose metabolism, accompanied by changes in the profile of metabolic hormones. The infection resulted in a phenotypic change in adipose tissue macrophages that was characterized by upregulation of alternative activation markers. Interleukin-13 (IL-13) activation of the STAT6 signaling pathway was required for the infection-induced attenuation of steatosis but not for improved glucose metabolism, whereas weight loss was attributed to both IL-13/STAT6-dependent and -independent mechanisms. Parasitic nematode infection has both preventive and therapeutic effects against the development of obesity and associated features of metabolic dysfunction in mice.**

The incidence of obesity has increased dramatically worldwide and has risen to an endemic level in the United States (1). Multiple factors may contribute to obesity, including genetic predisposition and environmental, socioeconomic, and behavioral factors. In addition, obesity is one of the key risk factors for many metabolic diseases, such as diabetes, steatosis, hypertension, and heart disease. Recent studies indicated that obesity is accompanied by chronic low-grade inflammation in adipose tissues, mainly due to accumulated inflammatory cells (Th1/Th17 cells, macrophages, etc.) (2). Inflammatory cells, together with adipocytes, release a variety of proinflammatory cytokines and chemokines, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and CCL2, that contribute to the development of metabolic syndrome and may exacerbate the consequences of obesity (3).

Gastrointestinal nematodes infect ~2 billion people worldwide, remaining the most prevalent of all chronic human infections. In developed countries, there is a decreasing incidence of helminth infection but a rising prevalence of certain types of autoimmunity, suggesting a relationship between the epidemiology of these diseases and inappropriate immune responses (4). Parasitic nematode infection induces a marked elevation of Th2 cytokines, including IL-4, IL-5, IL-13, and IL-25, that is linked to protective immunity against the infection (4). Many of these cytokines also have potent anti-inflammatory effects against various Th1/Th17-associated pathologies (5). In fact, nematode infection has been purported to have therapeutic effects in various autoimmune diseases (6), and several clinical trials are testing the therapeutic effects of nematode infection against Th1/Th17-associated diseases such as inflammatory bowel disease, allergy, and multiple sclerosis (Human Helminth Co-Infections Clinical Trials Database [www.niaid.nih.gov]).

Immunometabolism is an emerging field of investigation, and a major focus has been the contribution of proinflammatory cytokines and mediators to obesity and the disruption of metabolic homeostasis. The role of anti-inflammatory Th2 cytokines in this disorder, however, remains relatively unexplored. Given that obesity is associated with increased production of proinflammatory cytokines that contribute to insulin resistance, whereas nematode infections induce strong Th2 and T regulatory cell responses that can downregulate Th1/Th17-dependent immunity, we hypothesized that manipulating *in vivo* immune responses through parasitic nematode infection would be beneficial against obesity and the associated metabolic dysfunction. The present study used the gastrointestinal nematode parasite *Nippostrongylus brasiliensis* in mice with obesity to investigate (i) preventive and therapeutic effects of nematode infection against the development of obesity and associated metabolic dysfunction, (ii) molecular mechanisms that regulate infection-induced changes in body weight and glucose/lipid metabolism, and (iii) contributions of IL-13 and STAT6 to these mechanisms.

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## MATERIALS AND METHODS

**Mice and diet.** RIP2-Opa1KO mice with a pancreatic β cell Opa1 deficiency (RIP2-Cre *Opa1*$^{flox/-}$) and littermate controls (RIP2-Cre *Opa1*$^{flox/+}$) were generated as described previously (7). They were used as a genetic deficiency-induced model of obesity. Male C57BL/6 wild-type (WT) mice or mice with a deficiency of STAT6 (STAT6$^{-/-}$) or IL-13 (IL-13$^{-/-}$), at the age of 5 weeks, were fed a normal control diet (NCD) or given a high-fat diet (HFD) (60% of kcal from fat; Research Diets, New Brunswick, NJ) to induce obesity. These studies were conducted in accordance with the principles set forth in the *Guide for the Care and Use of Laboratory Animals* (8) and by the Beltsville Area Animal Care and Use Committee (protocol 10-005).

***N. brasiliensis* infection.** Infective, third-stage larvae of *N. brasiliensis* ($L_3$) (specimens on file [collection 81930] at the U.S. National Parasite Collection, U.S. National Helminthological Collection, Beltsville, MD) were propagated and stored at room temperature in feces-charcoal-peat moss culture plates until use (9). For infection, groups of mice were inoculated subcutaneously with 500 $L_3$ parasites (10). To determine if a parasitic nematode infection affects body weight gain from genetic deficiency, RIP2-Opa1KO mice were infected with *N. brasiliensis* at 10 weeks of age, before there was a significant difference in body weight between RIP2-Opa1KO mice and their littermates, and again at 17 weeks of age, to enhance the effect. To determine if a parasitic nematode infection affects HFD-induced body weight gain, mice were infected with *N. brasiliensis* once every 4 weeks, for a total of three infections, while being kept on the HFD or NCD throughout the experiment. To determine whether a parasitic nematode infection induces weight loss, obese mice were infected with *N. brasiliensis* after 14 weeks on the HFD.

**Glucose tolerance test.** An oral glucose tolerance test (OGTT) was carried out in mice after overnight fasting. Mice were administered glucose orally (1 g/kg body weight; 25% in saline) by gavage. Blood glucose levels were monitored from tail vein blood samples 0, 30, 60, and 120 min after administration of glucose.

***In vitro* glucose absorption by the intestine as measured in Ussing chambers.** Muscle-free segments of small intestine were mounted in Ussing chambers (11). Concentration-dependent changes in short-circuit current ($I_{sc}$) were determined for the cumulative addition of glucose to the mucosa side. Responses from all tissue segments exposed to glucose from an individual animal were averaged to yield a mean response per animal.

**RNA extraction, cDNA synthesis, real-time qPCR, and multiplex enzyme-linked immunosorbent assay (ELISA).** Total RNA was extracted with TRIzol reagent (Invitrogen, Grand Island, NY). RNA samples (2 μg) were reverse transcribed to cDNA by use of a First Strand cDNA synthesis kit (MBI Fermentas, Hanover, MD). Quantitative PCR (qPCR) was performed on an iCycler detection system. The fold changes in mRNA expression for targeted genes were relative to the respective vehicle groups of mice after normalization to the 18S rRNA gene. Primer sequences are listed in Table S1 in the supplemental material. Circulating levels of metabolic hormones in plasma samples were measured using a Bio-Plex Pro diabetes assay per the manufacturer's instructions (Bio-Rad, Hercules, CA).

**Oil red staining.** Tissue sections of liver were cut from frozen blocks prepared with dry ice plus acetone (Histoserv, Germantown, MD) and stored at −80°C. Slides were fixed in 10% formalin and incubated with 0.1 ml of 0.7% oil red O solution, washed with 85% propylene glycol, and then stained with hematoxylin. Slides were mounted with glycerin jelly and photographed for analysis.

**Immunofluorescence staining.** Tissue sections (8 μm) of epididymal fat were cut from frozen blocks prepared with dry ice plus acetone (Histoserv, Germantown, MD) and stored at −80°C. Slides were fixed in cold acetone for 30 min, blocked with 5% normal donkey serum in phosphate-buffered saline (PBS), and then incubated with anti-F4/80 (BioLegend, San Diego, CA) and anti-YM-1 (R&D Systems, Minneapolis, MN) antibodies overnight at 4°C. After being washed, the slides were stained with Dylight 488–donkey anti-rat IgG and Dylight 659–donkey anti-goat IgG (1:400; Jackson ImmunoResearch, West Grove, PA) for 1 h and then digitally photographed with a Nikon TE 2000-E microscope (Melville, NY). The images were taken by establishing settings for the samples from mice fed the NCD and using the same conditions to evaluate the samples from mice given the HFD and from infected mice. Comparisons were made only among slides prepared on the same day.

**Lipid extraction and analysis.** Tissue homogenates were prepared in lipid extraction buffer, shaken in the dark for 2 h, and centrifuged at 3,500 rpm for 10 min. The soluble part was transferred to a 1.5-ml tube and dried under vacuum for 30 min. The dried lipids were solubilized in triglyceride assay buffer by vortexing until the lipids were homogeneous. Levels of hepatic triglycerides were determined using a commercial triglyceride assay kit (Cayman Chemical, Ann Arbor, MI).

**Data analysis.** Agonist responses were fitted to sigmoid curves (Graphpad, San Diego, CA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Neuman-Keuls test to compare the responses and gene expression among the different treatment groups, or the Student *t* test to compare the difference between two groups. *P* values of $<0.05$ were considered significant.

## RESULTS

**Infection with *N. brasiliensis* attenuated body weight gain and improved glucose homeostasis in RIP2-Opa1KO mice.** A deficiency of Opa1 in pancreatic β cells results in disrupted glucose homeostasis, including hyperglycemia and glucose intolerance (7). While the underlying mechanism remains undefined, mice with this deficiency gradually gain more body weight than their age-matched littermates, beginning around 12 weeks of age. Infection with *N. brasiliensis* caused significantly less body weight gain in RIP2-Opa1KO mice than in uninfected ones but had no apparent effect on the littermates (Fig. 1A). Notably, *N. brasiliensis*-infected RIP2-Opa1KO mice had significantly lower fasting blood glucose levels and less glucose intolerance (Fig. 1B) than those of uninfected RIP2-Opa1KO mice. In contrast, *N. brasiliensis* infection did not affect the glucose metabolism of the littermates (Fig. 1B).

**Infection with *N. brasiliensis* attenuated body weight gain in mice fed a high-fat diet.** The effect of infection with *N. brasiliensis* on body weight gain was investigated further in an HFD-induced model of obesity. As expected, mice on the HFD gradually gained more body weight than those on the NCD (Fig. 1C). Notably, mice on the HFD that received the infection gained less weight than the control mice, and the difference was detectable as early as the 4th week on the diet. Obese mice on the HFD had high fasting blood glucose levels and were glucose intolerant, as expected (Fig. 1D). Compared with control mice on the HFD, mice receiving the infection appeared to have an improved glucose metabolism, as the blood glucose levels were lower at all time points examined, although the differences were not statistically significant. The overall food intake levels were comparable among the groups, with the exception that infected mice consumed less food than uninfected controls during the first 3 days after each infection (data not shown).

**Obese mice infected with *N. brasiliensis* showed significant body weight losses and decreased adipose tissue masses.** Subsequent experiments were carried out to determine whether nematode infection can induce body weight loss in mice with obesity. Mice were kept on the HFD for ~14 weeks and then infected with *N. brasiliensis*. At day 10 postinfection (p.i.), when all adult worms had been expelled, infected obese mice had lost nearly 13% of their body weight, despite continuation of the HFD (Fig. 2A). In another, separate experiment, infected obese mice had lost 9% ± 1%

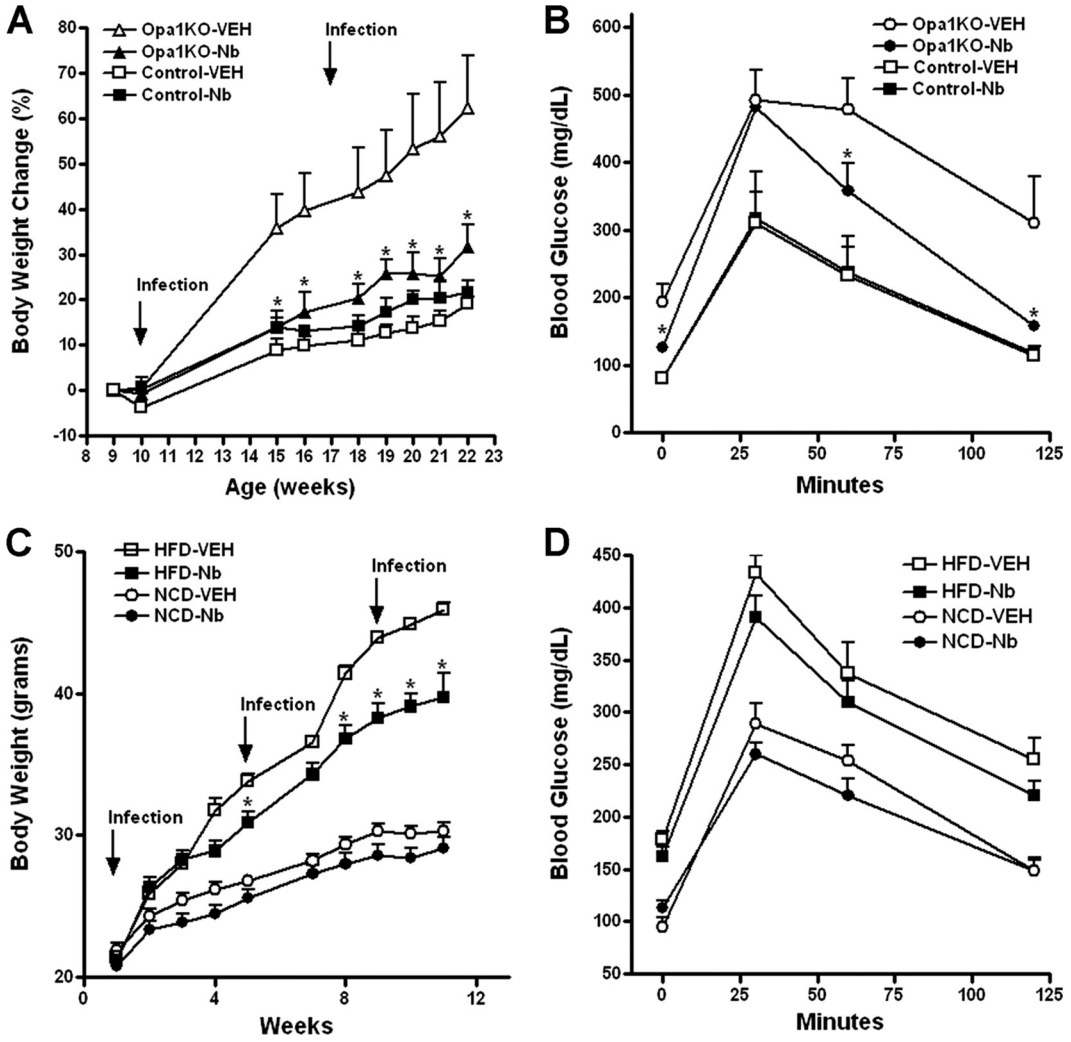


**FIG 1** Prior or concurrent infection with *N. brasiliensis* attenuated body weight gain and improved glucose homeostasis in RIP2-Opa1KO mice or C57BL/6 mice fed a high-fat diet. (A and B) RIP2-Opa1KO mice on normal control chow were infected twice with *N. brasiliensis* (Nb) or treated with vehicle (VEH). (C and D) C57BL/6 male mice were infected three times with *N. brasiliensis*, at weeks 0, 4, and 8, while being kept on a high-fat diet (HFD) or a normal control diet (NCD) for a total of 11 weeks. Body weight was monitored weekly, and an oral glucose tolerance test (OGTT) was performed 2 days before euthanasia. Data shown in line graphs are means ± standard errors of the means (SEM) and are representative of two independent experiments. *, $P < 0.05$ versus the respective Opa1KO-VEH group (A and B; $n = 5$ to 8 mice per group) or HFD-VEH group (C; $n = 10$ mice per group).

of their body weight at day 20 p.i., long after the worms were cleared. The body weights of lean mice also changed significantly, with a gain in uninfected and a loss in infected mice (Fig. 2A). Again, mice receiving infection consumed less food in the first 3 days p.i. and returned to a normal level of food intake thereafter (data not shown).

As expected, most of the major organs in obese mice were larger than those in lean mice. In obese mice, *N. brasiliensis* infection resulted in significantly decreased masses of epididymal (~20%) and brown (~50%) fat, as well as the liver, compared with those of uninfected obese mice (Fig. 2B and 3A), while the kidney and heart masses were not significantly different between the two groups. In lean mice, *N. brasiliensis* infection also decreased the mass of epididymal fat (Fig. 2B). Accordingly, obese mice had higher circulating levels of leptin than the lean mice, whereas *N. brasiliensis* infection drastically decreased the level of leptin in both obese and lean mice, consistent with a decreased adiposity and improved leptin sensitivity (Fig. 2C). Splenomegalies (values in grams) were observed in the infected obese (0.192 ± 0.007 versus 0.123 ± 0.007) and lean (0.114 ± 0.004 versus 0.084 ± 0.007) mice compared with the respective uninfected mice, consistent with an ongoing immune activation.

**Infection with *N. brasiliensis* ameliorated hepatic steatosis in obese mice.** Obese mice on the HFD had significantly enlarged livers accompanied by increased levels of hepatic triglycerides (Fig. 3A and B). Infection with *N. brasiliensis*, however, ameliorated hepatic steatosis in obese mice, as indicated by decreased liver masses and levels of hepatic triglycerides (Fig. 3A and B). The decreased lipid accumulation in livers of infected obese mice was confirmed by histological evaluation via oil red staining (Fig. 3C). In contrast, the infection did not affect the liver mass and triglyceride level in lean mice.

Gene expression of key enzymes and mediators involved in lipid metabolism was then analyzed. qPCR showed that there was

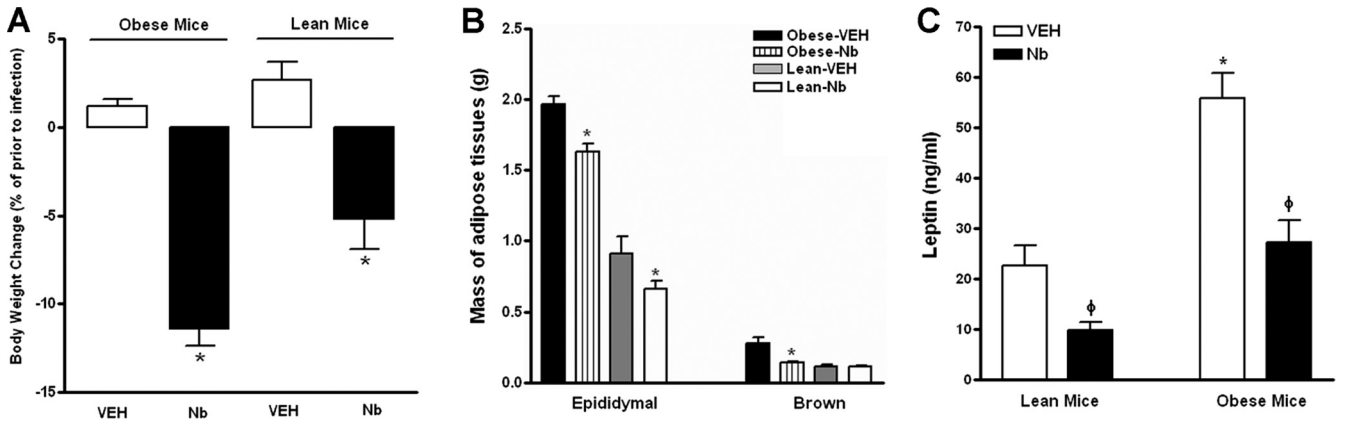


**FIG 2** Infection induced loss of body weight, loss of adipose tissue mass, and a decrease in the circulating level of leptin. Mice were fed an HFD or NCD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection. (A) Body weight changes at day 10 postinfection. (B) Weights of adipose tissues at euthanasia. (C) Circulating levels of leptin. Data shown in bar graphs are means ± SEM and were pooled from two independent experiments with 10 mice per group. *, $P < 0.05$ versus the respective VEH group (A and B) or lean-VEH group (C); ϕ, $P < 0.05$ versus the respective VEH group.

a generalized downregulation of the genes encoding key lipogenic enzymes in the liver (Fig. 3D) and epididymal fat (see Fig. S1 in the supplemental material), including *Fasn*, *Acly*, and *Acaca*, for obese mice compared with lean mice. Remarkably, *N. brasiliensis* infection resulted in an even further decrease in gene expression of these enzymes in obese mice but had no significant effect on lean mice (Fig. 3D; see Fig. S1). CIDEA is an important regulator of energy expenditure and lipid metabolism (12). Consistent with

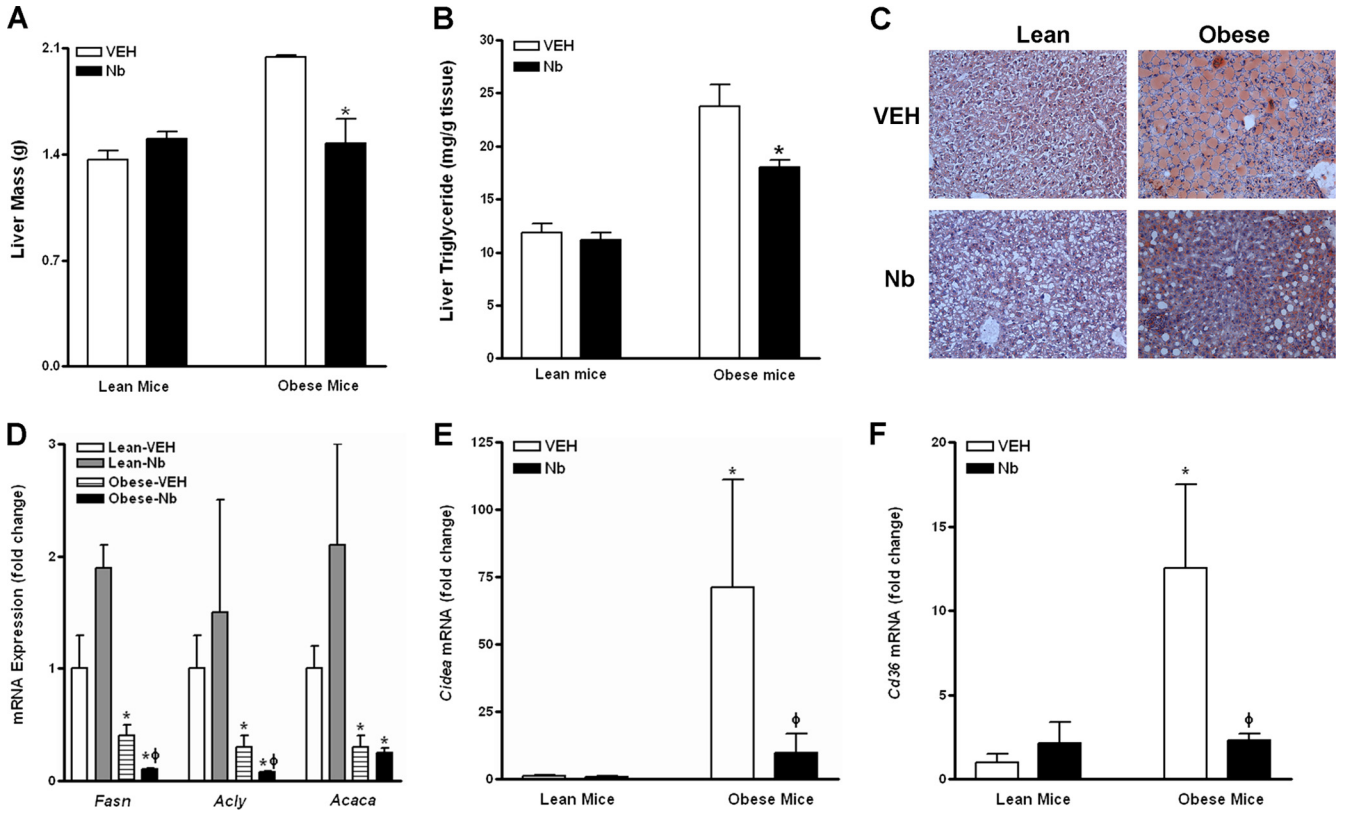


**FIG 3** Infection ameliorated hepatic steatosis in obese mice and was associated with changes in gene expression of key enzymes/mediators for lipid metabolism. Mice were fed an HFD or NCD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection. (A) Liver weights. (B) Levels of hepatic triglycerides. (C) Representative oil red staining of liver tissue sections. Tissues were collected for analysis of gene expression by qPCR. (D) Hepatic gene expression of *Fasn*, *Acly*, and *Acaca*. (E) Hepatic gene expression of *Cidea*. (F) Hepatic gene expression of *Cd36*. The fold changes were relative to the lean-VEH group after normalization to the 18S rRNA gene. Results shown in bar graphs are means ± SEM and are pooled data from (A and B) or representative of (D to F) two independent experiments with 5 mice per group per experiment. *, $P < 0.05$ versus obese-VEH group (A and B) or lean-VEH group (D, E, and F); ϕ, $P < 0.05$ versus obese-VEH group.

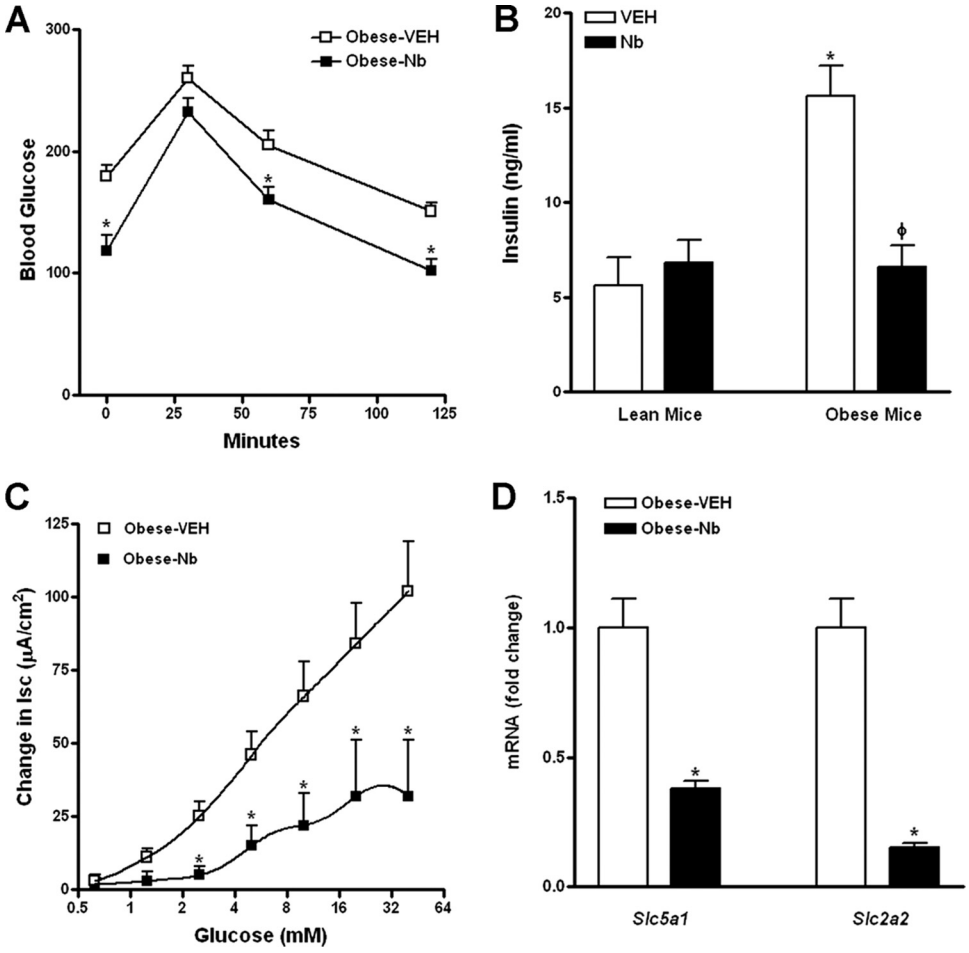


**FIG 4** Infection improved glucose homeostasis and decreased mucosal glucose absorption in obese mice. Mice were fed an HFD or NCD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection, except for the OGTT. (A) OGTT was performed on separate groups of mice at day 14 postinfection. (B) Circulating levels of insulin measured using the Bio-Plex diabetes assay. (C) Mucosae were mounted in Ussing chambers, and concentration-dependent changes in short-circuit current ($I_{sc}$) were determined for the cumulative addition of glucose to the mucosa side. (D) mRNA expression of the glucose transporters Slc5a1 and Slc2a2 in the small intestine was analyzed by qPCR. Data shown in line and bar graphs are means ± SEM and are representative of (A, C, and D) or pooled from (B) two independent experiments with 5 mice per group per experiment. *, $P < 0.05$ versus obese-VEH group (A and B) or lean-VEH group (C to E); ϕ, $P < 0.05$ versus the respective VEH group.

previous results (13), the hepatic gene expression of *Cidea* was significantly upregulated in obese mice. *N. brasiliensis* infection normalized hepatic *Cidea* expression of obese mice to the level in lean mice (Fig. 3E). CD36 in liver functions as a fatty acid (FA) plasma membrane transporter that takes up FA into hepatocytes (14). Gene expression of *Cd36* was upregulated in livers of mice fed the HFD but was inhibited significantly by *N. brasiliensis* infection (Fig. 3F). In contrast, gene expression levels of major hepatic enzymes critical for lipolysis or FA oxidation, including hepatic lipase, carnitine palmitoyltransferase 1a, and hydroxyacyl-coenzyme A dehydrogenase, were not significantly altered by the HFD or infection (data not shown).

**Infection with *N. brasiliensis* normalized glucose homeostasis in obese mice.** Obese mice fed the HFD had disrupted glucose homeostasis with significantly elevated fasting blood glucose levels as well as an inability to dispose of glucose efficiently (Fig. 1D and 4A). Notably, obese mice had normalized fasting blood glucose levels and disposed of glucose more efficiently after infection with *N. brasiliensis* (Fig. 4A). The circulating level of insulin in plasma was substantially elevated in obese mice. Notably, *N. brasiliensis* infection decreased the insulin levels of obese mice down to the levels of lean mice, suggesting a restoration of insulin sensitivity (Fig. 4B). We previously showed that enteric nematode infection induced a stereotypic decrease in glucose absorption by the small intestine. Indeed, intestinal mucosae from *N. brasiliensis*-infected obese mice had significantly attenuated responses to the addition of glucose to the luminal side of the jejunal mucosa (Fig. 4C). This was associated with a downregulated expression of *Slc5a1* and *Slc2a2*, encoding the key glucose transporters in the intestine (Fig. 4D).

**Infection with *N. brasiliensis* induced a potent Th2 cytokine response and alternative activation of macrophages in adipose tissue.** As expected, *N. brasiliensis* infection significantly upregulated the transcripts for major Th2 cytokines, including *Il4*, *Il5*, and *Il13*, while the *Tnfα* and *Il12p40* transcripts were downregulated in the small intestine in both obese and lean mice (see Fig. S2A in the supplemental material). In addition, the infection increased gene expression of *Il4* (from undetectable to detectable)

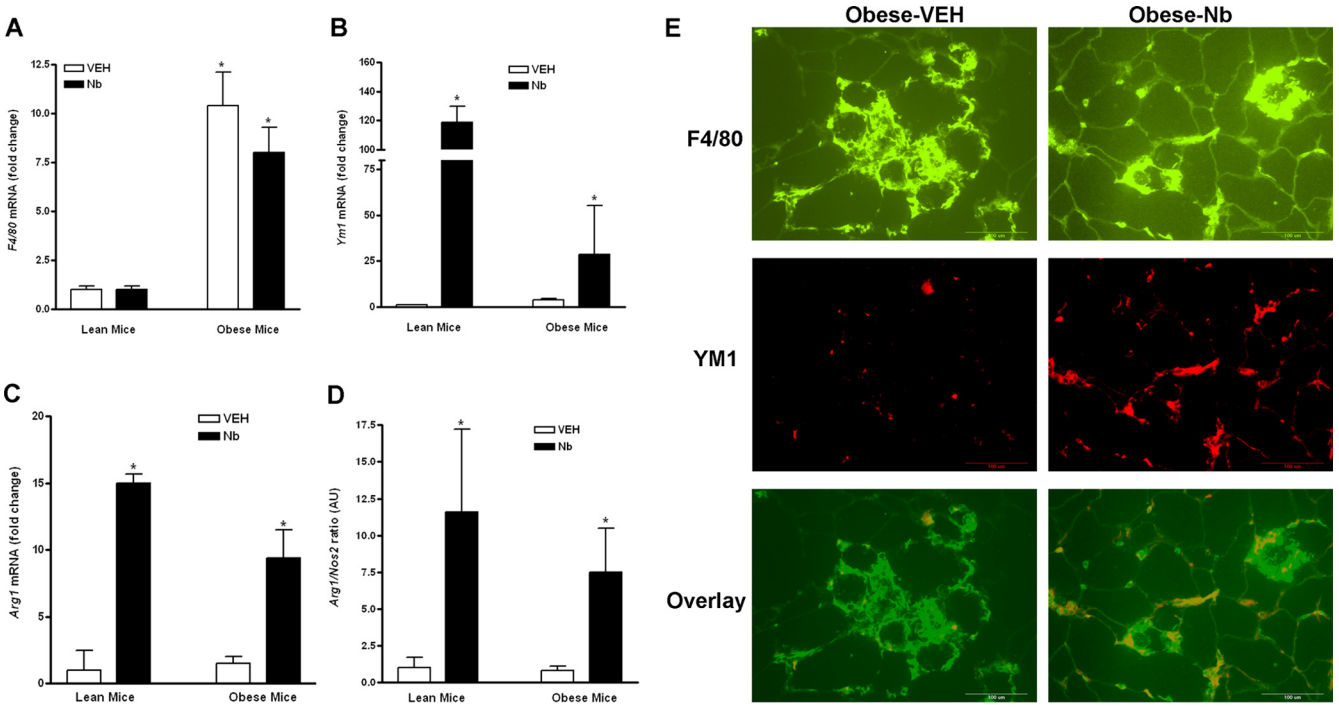


**FIG 5** Accumulation and alternative activation of macrophages in epididymal adipose tissue. Mice were fed an HFD or NCD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection. Gene expression of the macrophage markers F4/80 (A), Ym1 (B), and Arg1 (C) was determined by qPCR. The fold changes are relative to the lean-VEH group after normalization to the 18S rRNA gene. (D) Ratio of Arg1 to Nos2 gene expression as a marker of M2/M1 activation. AU, absorbance units. Data shown in bar graphs are means ± SEM. *, $P < 0.05$ versus the respective vehicle group. (E) Sections of epididymal fat from obese mice receiving *N. brasiliensis* infection (Nb) or vehicle were stained with anti-F4/80 (green) and anti-YM-1 (red). The images are representative of 5 animals in each group.

without affecting *Il5* and *Il13* expression in epididymal fat (see Fig. S2B), and it upregulated hepatic *Il5* but not *Il4* and *Il13* expression (see Fig. S2C). Furthermore, *N. brasiliensis* infection had no effect on HFD-induced gene expression of *Tnfα* and *Ccl2* in the liver and epididymal fat (see Fig. S2).

An accumulation of adipose tissue macrophages (ATM) with a dominant, classically activated (M1) phenotype contributes to development of insulin resistance in obesity (3). Indeed, *F4/80* gene expression was significantly upregulated in the epididymal fat of obese mice (Fig. 5A). Infection with *N. brasiliensis* did not change the expression level of *F4/80* but induced an alternative activation (M2) of ATM, as evidenced by upregulated expression of the M2 markers *Arg1* and *Ym1* (Fig. 5B and C). Even though inducible nitric oxide synthase (*Nos2*) was not altered by either the HFD or the infection (data not shown), increases in the *Arg1*/*Nos2* transcript ratio were consistent with greater M2 activation in epididymal fat from infected mice (Fig. 5D). This was further validated by immunofluorescence staining showing that more F4/80$^+$ YM-1$^+$ macrophages were visualized in epididymal fat from infected mice than in that from uninfected ones (Fig. 5E).

**Dependence of *N. brasiliensis* infection-induced beneficial effects on STAT6 and IL-13.** To determine whether the beneficial effects of *N. brasiliensis* infection on obesity and the associated metabolic dysfunction were dependent on IL-4/IL-13 activation of STAT6, we employed diet-induced obesity in STAT6$^{-/-}$ or IL-13$^{-/-}$ mice. STAT6$^{-/-}$ mice fed the HFD for 12 weeks gained significantly more body weight than STAT6$^{-/-}$ mice fed the NCD (40.6 ± 1.1 versus 33.1 ± 0.9 g), but less than the respective WT mice fed the HFD (45.3 ± 1.2 g), consistent with a previous report (15). *N. brasiliensis* infection caused ~5% body weight loss in obese STAT6$^{-/-}$ mice (Fig. 6A). Note that the infection-induced weight loss of STAT6$^{-/-}$ mice was significantly less than that of WT mice (12.3% ± 0.9%). Infection also resulted in significant mass losses of epididymal and brown fat (Fig. 6B), as well as improved glucose metabolism (Fig. 6C), in obese STAT6$^{-/-}$ mice, in a manner similar to that in WT mice. In contrast, infection did not alter the severity of hepatic steatosis (Fig. 6D) or the expression of key enzymes/mediators for lipid metabolism of obese STAT6$^{-/-}$ mice (Fig. 6E). It should also be noted that the infection-induced upregulation of M2 markers in the epididymal fat and intestines of WT mice was absent in obese STAT6$^{-/-}$ mice (Fig. 6F).

Unlike STAT6$^{-/-}$ mice, IL-13$^{-/-}$ mice gained body weight similarly to WT mice after being fed the HFD. *N. brasiliensis* infection caused ~3% body weight loss in obese IL-13$^{-/-}$ mice (Fig. 7A), which was not significantly different from that of STAT6$^{-/-}$ mice but was less than that of WT mice. Infection also reduced the masses of epididymal and brown fat (Fig. 7B) but did not ameliorate the hepatic steatosis in obese IL-13$^{-/-}$ mice (Fig. 7C). Again, the glucose metabolism was significantly improved in obese IL-13$^{-/-}$ mice after infection (Fig. 7D).

## DISCUSSION

The incidence of obesity has become endemic globally, yet the treatment options are extremely limited. Our current study showed that *N. brasiliensis* infection had both preventive and therapeutic effects experimentally against obesity and the associated

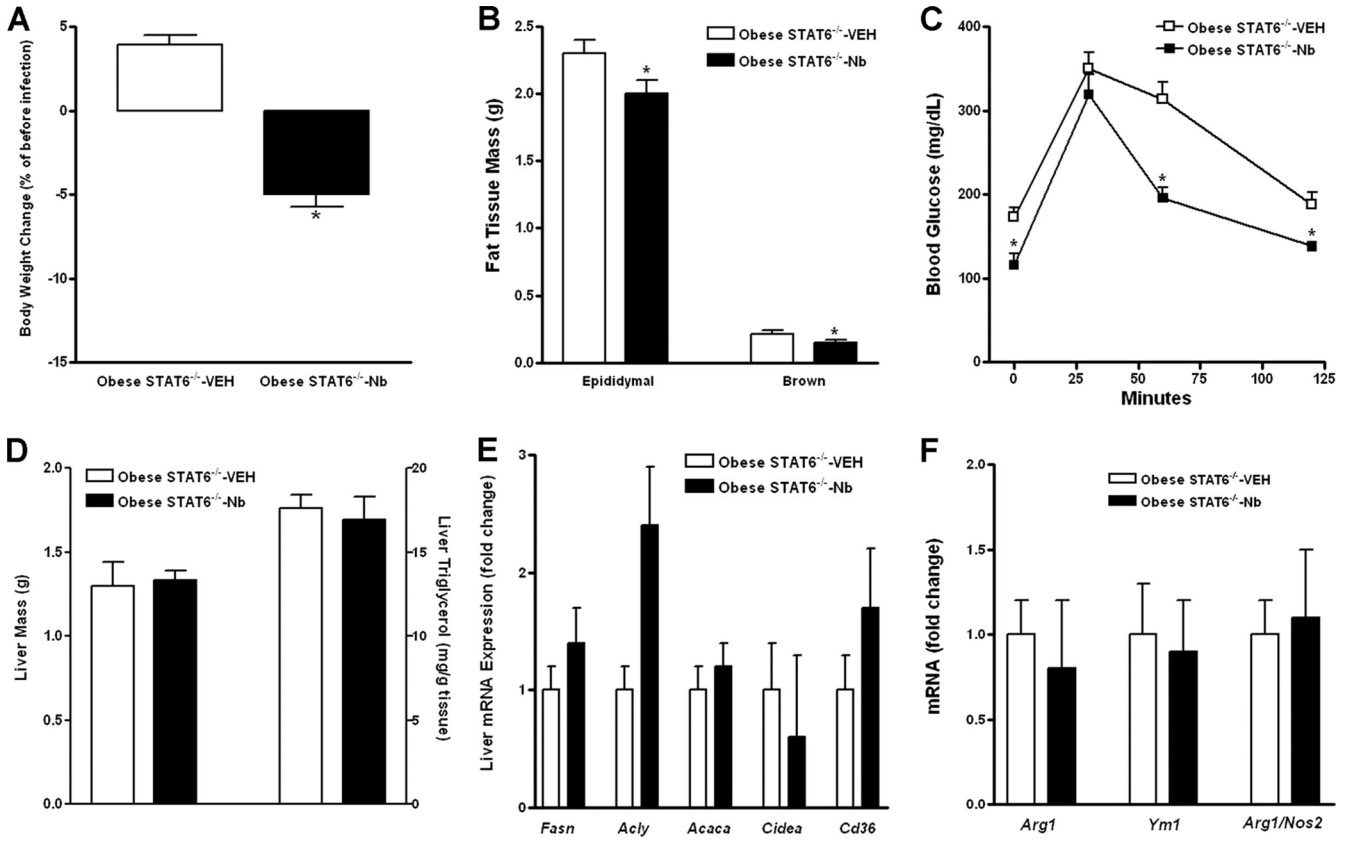


**FIG 6** STAT6 played distinct roles in *N. brasiliensis* infection-induced effects on body weight and metabolism. Mice with STAT6 deficiency ($STAT6^{-/-}$) were fed an HFD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection, except for the OGTT. (A) Body weight changes. (B) Masses of adipose tissues at euthanasia. (C) OGTT was performed on separate groups of mice at day 14 postinfection. (D) Liver masses and levels of hepatic triglycerides at euthanasia. (E) Hepatic gene expression of lipogenic enzymes/mediators. (F) Gene expression of the macrophage markers arginase I (Arg1) and Ym1 and ratios of Arg1 to Nos2 in the epididymal adipose tissue. Data shown in bar and line graphs are means ± SEM and are pooled from (A, B, and D) or representative of (C, E, and F) two independent experiments with 5 mice per group per experiment. *, $P < 0.05$ versus obese $STAT6^{-/-}$-VEH group.

metabolic dysfunction in mice. The beneficial effects were associated with induction of a polarized Th2 immune response, decreased intestinal glucose absorption, altered expression of the genes encoding major enzymes/mediators critical to lipid metabolism or uptake, modified circulating levels of metabolic hormones, and the accumulation of M2 macrophages in adipose tissue. IL-13 activation of the STAT6 signaling pathway was required for the infection-induced attenuation of steatosis but not for improved glucose metabolism. In addition, the effect of infection on body weight loss was attributed to both STAT6-dependent and -independent mechanisms (Fig. 8).

Enteric helminth infection induces a polarized Th2 cytokine response that is able to counterregulate the Th1/Th17 cytokine response (16, 17) and has been effective in experimental treatment of various autoimmune diseases. In our current study, infection with *N. brasiliensis* not only decreased body weight gain but also induced weight loss in established obese mice (Fig. 1). This is consistent with previous studies showing that rats infected with *N. brasiliensis* lost body weight (18). It is noteworthy that decreased food intake was only one of the factors responsible for the effects on body weight, as it occurred mainly in the first 3 days after infection. Administration of IL-4 was shown to increase energy expenditure and lipolysis in mice (19), which may also have contributed to the infection-induced weight loss observed in the current study. In addition, previous studies have linked inflammatory cytokines to obesity (20, 21), and blockade of TNF-α receptor-mediated signaling prevented obesity in rats (22). Thus, it is possible that nematode infection inhibits the production of inflammatory cytokines, thereby regulating body weight through immune modulation, as shown here by decreased gene expression of TNF-α and IL-12p40 in the small intestine after infection (see Fig. S2 in the supplemental material). To corroborate that, *N. brasiliensis* infection resulted in significantly less weight loss in obese $STAT6^{-/-}$ mice than in WT mice, despite the fact that the decreases in food intake were similar in both strains of mice (Fig. 6).

Dyslipidemia and hepatic steatosis are common in obese individuals, due to abnormalities in lipid metabolism. Infection with *N. brasiliensis* induced a significant amelioration of hepatic steatosis, indicating that nematode infection may also influence lipid homeostasis. Hepatic steatosis is caused by lipid accumulation within hepatocytes, mainly due to excessive lipogenesis. Our study showed that obesity itself resulted in decreased gene expression of lipogenic enzymes (Fig. 3), which may represent an adaptive response to limit the excessive lipogenesis in obese individuals. Nematode infection reduced gene expression of these enzymes

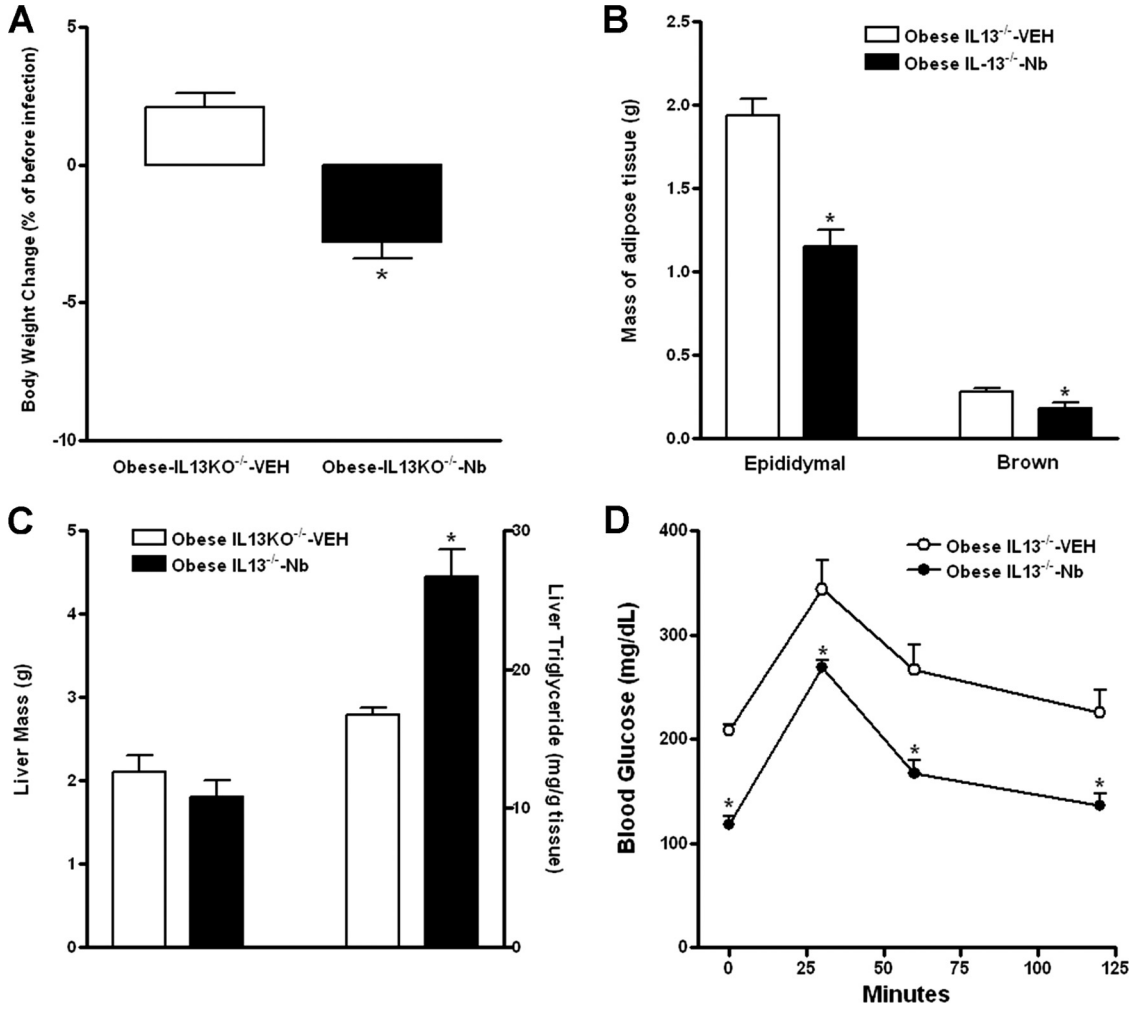


**FIG 7** IL-13 contributed to *N. brasiliensis*-induced weight loss and attenuation of hepatic steatosis. Mice with IL-13 deficiency (IL-13$^{-/-}$) were fed an HFD for 14 weeks, regrouped, and then infected with *N. brasiliensis* (Nb) or treated with vehicle (VEH). Mice were euthanized at day 10 postinfection. (A) Body weight changes. (B) Masses of adipose tissues at euthanasia. (C) Liver masses and levels of hepatic triglycerides at euthanasia. (D) OGTT was performed on separate groups of mice at day 14 postinfection. Data shown in bar and line graphs are means ± SEM and are from a single experiment with 5 mice per group. *, $P < 0.05$ versus obese IL-13$^{-/-}$-VEH group.

even further in the liver and adipose tissue (Fig. 3), two major organs for lipogenesis, representing a potential mechanism for the decreased adiposity and ameliorated hepatic steatosis observed in obese mice. CIDEA is a member of the cell death-inducing DFF45-like effector family and has emerged as an important regulator of energy expenditure and lipid metabolism (12). Mice with CIDEA deficiency were resistant to HFD-induced obesity, while the level of CIDEA expression was upregulated in the steatotic liver (13). The profound inhibitory effect of *N. brasiliensis* infection on hepatic CIDEA expression suggested that some of the beneficial effects of infection on regulation of lipid homeostasis work through this protein. Furthermore, infection downregulated the hepatic expression of CD36, an important regulator of fatty acid uptake for the liver (14). It is notable that the infection was unable to regulate these enzymes/mediators or to alter hepatic steatosis in mice lacking STAT6 (Fig. 6). In addition, hepatic triglyceride levels were even higher in mice deficient in IL-13 after the infection. Whether this was due to an IL-13 pathway defect resulting in a heightened Th1 immune response, which is known to disrupt the homeostasis of lipid metabolism, remains to be determined. Together, the data in our study suggest that *N. brasiliensis* infection regulates the synthesis and uptake of fatty acids, leading to decreased lipogenesis and, ultimately, reduced body weight via STAT6-depedent pathways.

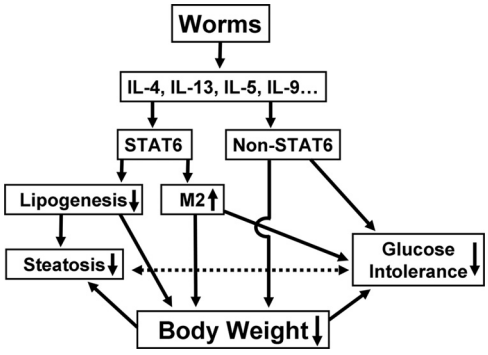


**FIG 8** Hypothetical model delineating the potential mechanisms by which worms modulate body weight and metabolism of the host.

The obesity-associated loss of glycemic control is driven primarily by insulin resistance and is accompanied by a dysfunctional hormonal regulation of metabolism. The elevated circulating levels of insulin found in obese humans and animals reflect a feedback mechanism to restore the control of glycemia by increasing insulin production. After *N. brasiliensis* infection, obese mice had a dramatic drop of insulin levels (Fig. 4), indicating that these mice had regained control of glucose metabolism and sensitivity to insulin. Enteric nematode infection induces characteristic changes in intestinal function, including a decrease in glucose absorption (9, 23). Accordingly, *N. brasiliensis*-infected obese mice had a nearly abolished epithelial response to glucose, accompanied by decreased expression of key glucose transporters in the intestine (Fig. 4), which might also provide benefits to glucose metabolism in obesity. Previous studies showed that nematodes or their secreted products can delay the onset of type 1 diabetes in mice (24, 25). The present study showed that nematode infection could effectively correct obesity-induced hyperglycemia and glucose intolerance, with or without accompanying body weight loss. The facts that *N. brasiliensis* is an acute infection that is expelled around 1 week after inoculation and that the worms do not become established in the small intestine after reinfection suggest that products produced by the worms do not have to be present continually to affect obesity.

Macrophages play an important role in obesity-associated tissue inflammation and metabolic syndrome (3). Depending on the cytokine microenvironment, macrophages undergo distinct pathways of activation, resulting in either the M1 or M2 phenotype (26). The ATM in obese individuals are of the predominant M1 phenotype and are linked to disrupted glucose homeostasis (3). Our present study showed that *N. brasiliensis* infection induced an M2-dominant phenotype without affecting the number of macrophages in adipose tissue (Fig. 5). Therefore, the beneficial effects of nematode infection on glucose homeostasis may be derived, at least in part, by the induction of the M2 phenotype.

Among the Th2 cytokines induced by nematode infection, IL-4 and IL-13 are the predominant effector molecules pivotal to host protective immunity. Engagement of receptors by IL-4 or IL-13 leads to activation of the STAT6 signaling pathway (16, 27). Of particular interest was the role of STAT6 in *N. brasiliensis* infection-induced effects on body weight and metabolic homeostasis. Specifically, the weight loss and attenuation of hepatic steatosis were dependent either partially or entirely on IL-13 activation of STAT6, whereas restoration of glucose homeostasis did not necessarily require STAT6 (Fig. 6). Accumulating evidence has pointed out a role of IL-4 induction of the M2 phenotype via STAT6 in restoring glucose homeostasis (15, 19, 28), yet our current study indicates that *N. brasiliensis* infection could still improve the glucose metabolism of obese mice in the absence of STAT6-mediated M2 development (Fig. 6), thereby excluding IL-4 as the major player. The underlying mechanisms remain to be identified, but it is known that nematode infection induces several Th2-related cytokines besides IL-4 and IL-13, such as IL-5, IL-9, IL-25, and IL-33 (17, 23). Administration of IL-33 led to reduced fasting blood glucose levels and improved glucose tolerance in ob/ob mice (29). Our ongoing studies indicate that exogenous IL-25 can lower fasting blood glucose levels and decrease glucose intolerance in obese mice fed an HFD, independent of STAT6 (Z. Yang et al., unpublished data). In addition, nematode infections induce various types of innate and adaptive immune cells, including macrophages, mast cells, eosinophils, and T and B cells, and many of those cell populations have documented roles, beneficial or detrimental, in obesity and the associated metabolic syndrome (5). It would be equally important to identify which factors and cell types contribute to the beneficial effects of *N. brasiliensis* infection on body weight and glucose homeostasis independent of IL-4/IL-13 activation of STAT6.

Taken together, our data clearly show the beneficial effects of nematode infection on obesity and the associated metabolic dysfunction. These effects can be attributed to several interrelated or independent events resulting from nematode infection: promotion of Th2 cytokine responses, alterations in intestinal function important for energy intake, inhibition of lipogenesis, and development of M2 macrophages (Fig. 8). All these combined effects led to a lean body and restoration of metabolic homeostasis. Our study suggests that nematodes or their products might be useful as therapeutic agents for the treatment of obesity and metabolic diseases.

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